REMARKS

Claims 33-64 are pending in the application. Claim 64 is withdrawn from consideration. Claims 65, 66 and 67 are new. Claims 33-63 are rejected.

Claim Rejection – 35 U.S.C. §112, Second paragraph

Claims 33-44, 48, 50-63 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention. The Examiner asserts that it is not clear how the presently claimed vaccines differ from the vaccines in the prior art, and in particular what structural or formulation difference distinguish the instant vaccines from those of the art. Applicants traverse and respectfully assert that Claim 33, now amended, recites a method employing an antigenic preparation that is not disclosed or suggested in the art. Nowhere in the cited prior art is described a vaccine comprising a split influenza virus antigen preparation and a surfactant that is able to induce, after a single intranasal dose, an immune response of a magnitude that meets international regulatory requirements, and in particular European Union official criteria, for influenza vaccines. Applicants assert that Claim 33 is definite in that it recites structure pertaining to the influenza antigen preparation (split) and a surfactant. Without being bound by theory, Applicants believe that the surfactant may stabilize the heamagglutinin component of the split influenza preparation thereby contributing to the successful immunization after only a single, intranasal administration. Whatever the reason, Claim 33, as amended, particularly and distinctly claims what Applicants believe to be their invention.

Claims 34 and 35 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention. The Examiner asserts that the phrase "for the or all" is not clear. Claims 34 and 35 have been amended to remove the offending language.

Claims 38-42 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention. The Examiner asserts that there is insufficient antecedent basis for "formulation" in Claim 33 upon which Claim 38 depends. Claim 38 is hereby cancelled as redundant with amended Claim 33. Accordingly, this rejection is now moot.

Claim 39 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which the applicant regards as his invention. Applicants have amended the claim as suggested by the Examiner.

Claim 43 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention. Applicants have amended the claim as suggested by the Examiner. In addition, Applicants have added new Claim 65, depending from Claim 43, wherein the cholic acid or derivative is sodium deoxycholate.

Claims 44, 48 and 61 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention. The Examiner states that use of the relative term "low" renders the claim indefinite. Applicants have amended the claim to clarify the meaning of "low". In particular, Claim 44 now recites that the haemagglunitin content is not more than about 30 μg per influenza strain included in the antigenic preparation. Claim 45 is hereby cancelled as redundant to amended Claim 44. Claims 46 and 47 have been amended in a manner similar to Claim 44. Claims 48, 49, 50 and 61 have been similarly amended.

Claim 49 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as his invention. Claim 49 has now been amended according to the Examiner's suggestion. Finally, new Claim 66, depending from amended Claim 49, has been added further limiting the volume of the single dose.

Claim Rejection – 35 U.S.C. §112, First paragraph

Claims 33-63 are rejected under 35 U.S.C. § 112, first paragraph, because the specification, while being enabling for methods wherein two or more administrations of the indicated antigen preparations or where a single administration of the preparations disclosed in examples 4 and 5 of the application are provided to a patient such that an immune response meeting the international regulatory requirements for influenza vaccines are met, does not reasonably provide enablement for methods where any single administration is made. Applicants traverse and respectfully assert that the instant claims, as amended, are fully enabled by the specification. As admitted by the Examiner, at least

two different vaccine preparations (as set forth in Examples 4 and 5), both of which fall within the scope of the instant claims, induced immune responses after a single, intranasal dose, that were equivalent to responses induced by a commercially available, parenteral influenza vaccine. However, the Examiner discounts the experiments in Example 6 demonstrating the ability of intranasal formulations with even lower doses of influenza antigens to induce significant serum antibody and mucosal IgA responses. Applicants respectfully state that, for several reasons, these data would be viewed favorably by those skilled in this art as fairly representative of the outcomes expected in humans. Firstly, the Examiner's attention is drawn to the fact that within the experiment, comparisons are made between vaccines administered intranasally and a vaccine administered parenterally. In all cases, the mucosal IgA response of the intranasally administered vaccine is superior to the parenteral vaccine, even at the lowest antigen doses (see figures 2 and 4) and without added surfactant or immunostimulant. Moreover, the addition of a surfactant (laureth 9) and in some cases an additional immunostimulant boosted serum antibody responses to nasally administered vaccines to levels not statistically lower than serum antibody responses induced by parenteral administration of equivalent or greater amounts on influenza heamagglutinin (see figures 1 and 3). Accordingly, these experiments suggest to one skilled in this art that similar improvements over responses induced with conventional, parenterally administered vaccines, can be achieved by carrying out the claimed methods.

Furthermore, Applicants provide herewith a reference (Bachmayer et al. Develop. Boil. Standard 28:336-339 (Karger, Basel 1975)) demonstrating the utility of data generated in mice to predict outcomes in man. Scientists skilled in this art will usually conduct initial *in vivo* experimentation in mice, and will take the results of these experiments as at least suggestive of the likely outcome in humans. The Bachmayer paper affirms this conclusion in the context of evaluating influenza vaccine potency and is therefore highly relevant to issue at hand. In view of the skill and usual practices in this art, there is no reason for the Examiner to doubt the objective truth of the Applicants' assertions (see In re Marzocchi, 439 F2d 220 (CCPA 1971) ("The first paragraph of § 112 requires nothing more than objective enablement. How such a teaching is set forth, either by the use of illustrative examples or by broad terminology, is of no importance. As a matter of Patent Office practice, then, a specification disclosure which contains a teaching of the manner and process of making and using the invention in terms which correspond in scope to those used in describing and defining the subject matter sought to be patented

must be taken as in compliance with the enabling requirement of the first paragraph of § 112 unless there is reason to doubt the objective truth of the statements contained therein which must be relied on for enabling support.").

In addition, Applicants provide herewith a declaration under 37 C.F.R. § 1.132 providing the results of the experiments set forth in Example 7. These data demonstrate that for all intranasal, single dose vaccines tested (see Table 6), at least one of the European Union official criteria are met for all strains of influenza virus tested. It should be noted that even the lowest dose of haemagglutinin (7.5 μg per strain) without immunostimulant, the criteria were met. These results are entirely consistent (as expected) with the results obtained in experimental animals (see instant Example 6 and the discussion above).

Accordingly, Applicants have successfully demonstrated a method to achieving significant prophylaxis against influenza infection or disease by administration of a single dose of a composition comprising a split influenza virus antigen preparation and a surfactant. Moreover, prophylaxis was achieved at several doses of haemagglutinin, including doses as low as 7.5 μg per strain. Applicants therefore respectfully assert that the instant claims, as amended, are fully enabled and request withdrawal of the enablement rejection.

Claim 47 is further rejected because the teachings in the application do not provide evidence that dosages of 7.5 μg HA in the absence of an immunostimulant would be effective for the induction of the requisite immune responses. For the reasons stated above, and in view of the declaration submitted herewith under 37 C.F.R. § 1.132, Applicants traverse and respectfully request withdrawal of the rejection.

Claims 33-63 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement. The Examiner recites from the M.P.E.P. and asserts that the instant specification does not provide a large enough number of working examples in order to support the full scope of the claimed genus due to the conflict between the teachings of the prior art and the teachings of the present invention (in mouse models). Applicants respectfully traverse and assert that, as described in the instant specification and as set forth in the declaration under 37 C.F.R. § 1.132 provided herewith, they have provided a significant number of working examples exemplifying a broad portion of the claimed genus. Moreover, Applicants have amended the claims to provide the formula and/or structural limitation that the Examiner requested.

Applicants thus provide far more than "a single working example", and therefore respectfully request withdrawal of the written description rejection.

Claim Rejection – 35 U.S.C. §102

Claims 33-38, 44-48, 50, 51, and 55-59 are rejected under 35 U.S.C. § 102(b) as being anticipated by Oh et al., Vaccine 10(8): 506-11. The Examiner admits that the cited reference 1) does not teach the relationship of the results achieved by the administration with respect to international standards and 2) teaches that multiple doses are administered. Nonetheless, the Examiner asserts that the Oh et al. reference anticipates the instant claims "because the claims do not exclude the administration of additional dosages, and because the Applicant has not distinguished the 'one-dose' vaccines from that used by Oh". Applicants respectfully traverse and state that in view of the instant amendments, the claims are not anticipated by Oh. As amended, the claims require that after single intranasal dose of a split influenza virus antigen preparation comprising a surfactant, one or more of the European Union official criteria for influenza vaccines are met. In contrast, Oh et al. teach a vaccination regimen requiring four administrations at one week intervals. Nothing in Oh teaches or even suggests that a significant, protective response could be induced by a single, intranasal administration. Moreover, Oh does not describe or suggest the inclusion of a surfactant in the vaccine formulation. Since Oh does not teach each and every limitation of the instant claims, Oh cannot anticipate those claims. Applicants therefore respectfully request withdrawal of the rejection.

Claims 33-38, 43-49, 51, and 55-58 are rejected under 35 U.S.C. § 102(a) as being anticipated by the teachings of Gluck et al., J. Virol. 73(9):7780-7786. Applicants traverse and repeat the arguments provided above regarding the Oh et al. reference. Gluck's vaccine comprises a virosomal influenza vaccine and requires the use of a mucosal adjuvant (see the abstract: "the use of HLT as a mucosal adjuvant after a single spray vaccination is necessary to obtain a humoral immune response comparable to that with parenteral vaccination."). Applicants respectfully point out that, although Gluck teaches a formulation comprising 7.5 μg HA without adjuvant, Gluck's "complete vaccination" requires two doses of this formulation and thus administration of 15 μg HA (see Gluck, Table 1, Vaccine group B) to achieve the desired effect. Gluck admits that this formulation is inferior to others tested (see page 7783, column 1). Moreover, Gluck admits that a single, intranasal dose of his vaccine is inadequate (see page 7785, column 1: "Our investigations

showed that two nasal applications were significantly better than one application with double antigen and adjuvant doses."). The instant claims, as currently amended, require that a single, intranasal dose of vaccine achieve a significant level of immunity not seen with less than two doses of the Gluck vaccine. Accordingly, Gluck does not teach or suggest the invention embodied by the instant claims, and therefore the 35 U.S.C. § 102 rejection should be withdrawn.

Claim Rejection – 35 U.S.C. §103

Claims 38-40, and 52-54 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Oh or Gluck as applied above, and further in view of Friede et al. (WO 99/52549). Applicants traverse and respectfully state that WO 99/52549 was published on October 21, 1999, after the earliest priority date of the instant application (September 24, 1999, based on GB 9922700.1 and GB 9922703.5). Accordingly, WO 99/52549 is not prior art to the instant application and therefore request withdrawal of the obviousness objection.

Claims 38-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Oh or Gluck in view of Friede as applied to claims 38-40, and 52-54 above, and further in view of either Baker et al. (U.S. 6,506,803) or Morein et al. (U.S. 5,679,354). Applicants traverse and respectfully repeat the arguments above regarding the Oh, Gluck and Friede references. In addition, Applicants assert that while Baker and Morein may suggest the use of certain surfactants for certain purposes, neither suggests to one skilled in this art, either alone or in combination with the Oh or Gluck references, a method of successfully inducing a protective immune response (as measured by European Union official criteria) against influenza infection by administering a single, intranasal dose of a vaccine containing these surfactants. There is simply no suggestion in the cited art that single dose immunization against influenza could be achieved at all, let alone with a vaccine containing a surfactant. Accordingly, Applicants respectfully request withdrawal of this rejection.

Claims 50, and 61-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over either Oh or Gluck as applied to claims 33-36, 38, 43-49, 51, and 55-58 above, and further in view of either Baum et al. (U.S. 3,874,381) or Weinstein et al. (U.S. 5,437,267). Applicants respectfully traverse. Oh and Gluck have been discussed (*supra*). Nothing in either Baum or Weinstein suggests that the use of an intranasal device could achieve

significant protection against influenza after only a single dose of vaccine. Accordingly, Applicants respectfully request withdrawal of this rejection.

Claims 60-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over the teachings of Barrett et al. (WO 00/47222) in view of either Baum or Weinstein as applied against claims 50, and 61-63 above. Applicants traverse, and respectfully point out that the primary reference (Barrett et al., WO 00/47222) was published on August 17, 2000, well after the earliest priority date of the instant application (September 24, 1999, based on GB 9922700.1 and GB 9922703.5). Accordingly, WO 00/47222 is not prior art to the instant application and therefore request withdrawal of the obviousness objection.

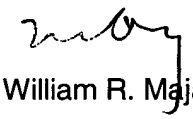
Claims 59-63 are rejected under 35 U.S.C. 103(a) as being unpatentable over the teachings of Chatfield (WO 97/16208) in view of Barrett, Baum, Weinstein as applied against claims 61-63 above. Applicants traverse. Chatfield's kits each require the presence of "an effective adjuvant amount of a chitosan" (see WO 97/16208, page 3, 4th full paragraph). The reference makes clear that an effective adjuvant amount is that amount that amount that results in "good systemic and local immune responses" (page 3, first full paragraph). Accordingly the reference requires the inclusion of an immunostimulant, a component specifically excluded from Claim 59 and claims dependent thereon. Moreover, none of the reference suggest that an aerosol delivery device for intranasal delivery (as taught in Baum and Weinstein) could be combined with the teachings of Chatfield to achieve the invention claimed in Claims 60-62 and claims dependent thereon. That is, none of the cited references, either alone or in combination, suggests that significant immunity could be achieved after a single dose of a split influenza antigen preparation in the presence of a surfactant. Accordingly, none of the references could suggest or render obvious the kits claimed in Claims 59-63.

CONCLUSION

In view of the foregoing amendments and remarks, Applicants respectfully submit that the subject application is in condition for allowance. Moreover, Applicants request reconsideration of the Election/Restriction Requirement in view of the discussion of the prior art. In particular, in view of the foregoing remarks, Applicants respectfully assert that the special technical feature of the invention, namely the provision of a vaccine that after a single dose is capable of inducing a significant protective immune response, distinguishes their invention over the art. Since the current claims, as amended, and in particular linking Claim 33, are distinguished over the art, Applicants state that the claims are properly linked and that therefore unity of invention is satisfied.

If the Examiner has any remaining objections or concerns, the Examiner is respectfully requested to contact Applicants' undersigned attorney to resolve such issues and advance the case to issue.

Respectfully submitted,



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POTENCY OF INFLUENZA VACCINES : MOUSE PROTECTION EXPERIMENTS IN CORRELATION TO FIELD STUDIES IN MAN

H. Bachmayer, E. Liehl and G. Schmidt

ABSTRACT

The seroconversion rates have been studied following vaccination of human volunteers with two commercial influenza vaccines.

Vaccine A did not give a significant increase of hemagglutination-inhibition titers. Vaccine B, on the other hand, raised the titers 2- to 8- fold, depending on the pretiters of the individuals.

The potency of the same vaccines has been tested using mouse protection experiments : vaccine B gave significantly better protection rates, as measured by survival as well as by reduction of lung lesions.

These results give additional evidence that the use of mouse protection experiments for the evaluation of different influenza vaccines is meaningful.

* * *

Measurement of the hemagglutinin content is the technique usually employed for the standardization of influenza vaccines. These tests are not accurate and for vaccines containing adjuvants the method is not applicable. The usual criteria for potency testing of such vaccines are the formation of hemagglutinin-inhibiting antibodies (HI) in man or animal and protection experiments in mice (2). Potency testing using mouse protection experiments has the advantage that it encompasses all factors of the immune response to a vaccine. A correlation between the seroconversion rates in man and the mouse protection test would be desirable for the standardization of vaccines and also for the experimental evaluation of antigen preparations.

The outcome of two influenza vaccination campaigns performed in two branches of our company, using two different commercial vaccines, prompted us to check the correlation of seroconversion rates in man and mouse protection rates.

MATERIAL AND METHODS

Vaccines. Vaccine A (1972/73) contained 17500 HA-units (700 IU) of A_2 virus strains (Singapore plus Hong Kong) and 7000 HA-units (280 IU) of B-type influenza virus per human 0.5 ml dose.

This vaccine contained $Al(OH)_3$ as adjuvant.

Vaccine B (1973/74) contained 400 IU A/England/72 plus 240 IU B/Berkeley/1/71 per 0.5 ml dose.

Vaccination schedules. Before vaccination, blood was taken from all volunteers to determine their pretiters. Single-dose syringes were used for vaccination. Four weeks later a second blood sample was obtained and the HI titers determined. A total of 47 and 52 persons received vaccine A and B, respectively. The distribution of sex and age for the two groups was comparable.

Determination of hemagglutinin-inhibition titers (HI) was performed in thermal and periodate inactivated antisera using automatic microtiter equipment (Cooke Engineering Co.). Hong Kong 68 (X-31) virus was used as antigen.

Mouse protection experiments. Female white mice (NMRI strain), weighing 20 to 24 g were used. The mice were immunized by intraperitoneal injection of 0.25 ml vaccine dilution containing 0.1% $Al(OH)_3$ (3). Thirty mice were used per group.

Twenty days after immunization, mice were challenged by aerosol infection (1) with 10 LD_{50} (according to Reed and Muench) of mouse-adapted X-31 virus. Mortality and lung lesions were scored up to day 13 after challenge and compared to untreated control groups. Protection rates were calculated from both criteria.

RESULTS AND DISCUSSION

Figures 1 and 2 demonstrate the HI titers obtained before and after vaccination with the two vaccines studied. Pretiters in group B were found to be higher than the corresponding group A titers. Mean HI titers following vaccination were nevertheless higher after vaccination with vaccine B than with vaccine A.

Vaccine B shifts the HI titers significantly towards higher titers; vaccine A has practically no influence.

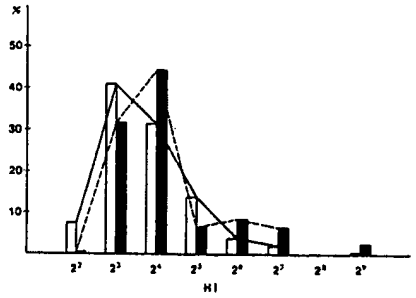

Fig. 1. Distribution of hemagglutinin-inhibiting titers (HI) before (open bars, ——) and 4 weeks after (black bars, ---) vaccination with vaccine A.

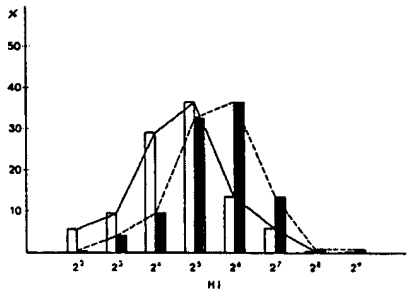

Fig. 2. Distribution of hemagglutinin-inhibiting titers (HI) before (open bars, ——) and 4 weeks after (black bars, ---) vaccination with vaccine B.

Figure 3 shows the seroconversion rates for immunization with the two vaccines : the rise in titers caused by vaccine B is statistically significant ($p < 0.05$); vaccine A has no significant effect on the HI titer distribution.

The results of the mouse protection experiments are summarized in Figure 4. The beneficial effect of three vaccine dilutions, all containing $Al(OH)_3$ as an

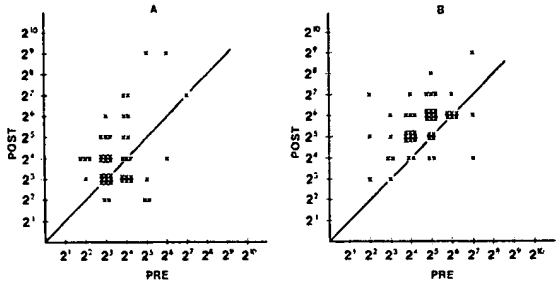

Fig. 3. Individual HI titers before and after vaccination with vaccines A and B.

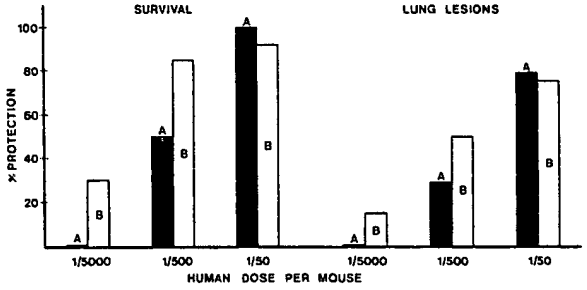

Fig. 4. Mouse protection experiments with vaccine A (black bars) and vaccine B (open bars).

adjuvant, was compared for the two vaccines studied. Protection rates for survival as well as reduction in the percentage of lung lesions are better with vaccine B than with vaccine A. The statistical significance was calculated for the survival rates at the three vaccine dilutions used : the differences are highly significant ($p<0.001$) at the highest vaccine dilution (1/5000 human dose per mouse) and significant ($p<0.01$) at the medium dilution (1/500 human dose per mouse). No significant difference was found after application of 1/50 human dose per mouse, giving almost complete protection with both vaccines.

Direct evaluation of influenza vaccine quality in man by measurement of the protection rates during an epidemic cannot be carried out for every batch or new type of vaccine in study. The significance of the HI seroconversion rate as parameter of influenza vaccine potency in man is well established (2), and hence any correlation between the more rapid mouse protection experiments and this parameter are of considerable importance. The data presented in this communication allow a direct correlation of the seroconversion rates in man and of mouse potency tests for two influenza vaccines. This correlation allows - in a relatively simple way - the comparative potency testing of different influenza vaccines. This can be useful for the development of new vaccines and for the control and standardization of influenza vaccines.

Acknowledgments

Statistical calculations were done by Mr. K. Neumann. We wish to thank Dr. H. Knapp and Mrs. K. Haberl for performing the vaccinations. The skilful technical assistance of Miss I. Falk and Mrs. H. Wege is gratefully acknowledged.

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